

(~19-52 mol%). We found that, at 24°C, the initial rate of CA4P release varies with cholesterol content in an alternating manner, showing a local maximum at 20.0, 22.2, 25.0, 32.8, 40.0 and 50.0 mol%. These cholesterol mole fractions are in excellent agreement with the theoretically predicted cholesterol critical mole fractions (Cr) for maximal superlattice formation. Similar results were obtained at 37°C. The faster release of CA4P at Cr can be explained in terms of the sterol superlattice model. At any given cholesterol mole fraction, sterol superlattice regions always coexist with non-superlattice regions. At Cr, the interfacial areas between superlattice and non-superlattice domains reach a local maximum (Venegas et al. (2007) *J. Phys. Chem. B.*, 111, 5180-5192). Since the interfacial areas have more void space and volume fluctuations, CA4P can leak out more easily at Cr. These results may help to optimize the liposomal CA4P formulation to achieve higher efficacy for cancer treatment.

1794-Pos Board B704

Effect of Amide-Linked Acyl Chain Length on the Sphingomyelin-Cholesterol Interactions

Shishir Jaikishan, Christian Ijäs, Thomas K.M. Nyholm, J. Peter Slotte.

Sphingolipids are important component of eukaryotic membrane and are crucial for several physical as well as biological processes in cells. Sphingolipids play key role in the lateral domain formations which further assists in cell signalling and various significant cellular phenomena. Even though sphingolipids display an extensive diversity in their structures both in the head group and hydrophobic region, sphingosine (D-erythro-2-amino-trans-4-octadecene-1, 3-diol) is the prevalent backbone in most mammalian sphingolipids, including sphingomyelin (SM). The aim of this study was to examine how variation in the chain length in SM affect their bilayer properties as well as their interactions with cholesterol. We have synthesized SM analogs with different amide-linked acyl chains (14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 22:0 and 24:0). The main transition temperature and the degree of acyl chain order in pure SM bilayers was determined from the steady-state anisotropy of diphenylhexatriene. Ordered domain formation and sterol interaction with SM analogs was determined from the quenching susceptibility of diphenylhexatriene and cholestatrienol. Sterol affinity to fluid bilayer membranes containing saturated SM analogs was determined using a cholestatrienol equilibrium partitioning assay. We found that all SM analogs formed ordered domains in a fluid bilayer. Sterol was always included in these ordered domains, albeit to a varying degree. The thermostability of the ordered domains was remarkably similar, even though the SM acyl chain length varied (16-24 carbons). Only 14:0 and 15:0 SM formed less thermostable ordered domains. We hope our results will enable us to better understand the behavior of asymmetric SM molecules in complex membranes, and to predict how they influence properties of biological membranes.

1795-Pos Board B705

Combined Fluorescence and Brewster Angle Microscopy of Dmpe/D-Cholesterol Mixed Langmuir Films

Fanindra Bhatta, Pritam Mandal, Edgar E. Kooijman, David W. Allender, J. Adin Mann, Jr., Andrew J. Bernoff, Elizabeth K. Mann.

The phase diagrams of DMPC/Cholesterol mixed Langmuir films have long been studied as an example of the critical mixing/demixing transition, with possible applications to membrane domains (rafts) in the much more complex biomembrane system. However, most of these studies have been performed with fluorescence microscopy, requiring the addition of a fluorescent probe, which can in principle affect the phase diagram. Here we combine Brewster angle and fluorescence microscopy on the same trough which allows us to verify if identical domain patterns are seen by the two techniques. Comparing Brewster angle results with and without fluorescent probe provides insight into the role of the probe on domain morphology and the surface pressure dependence of domain formation. We also compare the line tensions of the domains measured with and without the fluorescent probe and draw conclusions on the potential problems encountered when using fluorescent probes.

1796-Pos Board B706

Lipid Lateral Diffusion and Hydrocarbon Chain Order

Olivier Soubias, Walter E. Teague, Klaus Gawrisch.

Recent advances in molecular simulations have stimulated new discussions on the molecular mechanisms of lateral diffusion of lipids in fluid bilayers. To enable a deeper comparison between theory and experiments, we conducted a systematic study of lateral diffusion rates for a series of phosphatidylcholines with 14, 16, 18 and 20 hydrocarbons per chain over the temperature range from 10 to 60°C. Bilayers of the pure lipids as well as binary mixtures with 30 mol% cholesterol were investigated. Diffusion coefficients were measured by ¹H MAS NMR with application of pulsed magnetic field gradients. All lipids had saturated, perdeuterated sn-1 chains that enabled measurement of chain order parameters by ²H NMR. From the average chain order parameters effective hydrophobic thicknesses of bilayers and lateral areas per lipid molecule were calculated. This enabled

a quantitative comparison of experimentally determined and calculated lateral diffusion rates as a function of chain length, cholesterol content and temperature using a free-volume model of lateral diffusion. The implications of this comparison for models of lateral lipid diffusion will be discussed.

1797-Pos Board B707

Interactions Between Carboxyl Terminated Nanoparticles and Phase Separated Lipid Monolayers

Benjamin L. Stottrup, Ravi Tavakley, Sylvio May, Matthew P. Goertz, Howard L. Brockman.

Line tension between coexisting liquid phases in phospholipid/cholesterol model membranes is an extremely rich area of study because of its application to the raft hypothesis. Monolayers present an ideal system to study fundamental interactions in lipid raft forming compositions. We have previously described a method to extract line tension using Fourier analysis of domain boundaries. Here we present observations of the influence of 20 nanometer carboxyl terminated polystyrene nanoparticles on the line tension in raft forming monolayers. Combining the novel experimental approach developed by Brockman and colleagues [*Anal. Chem.* (2006) 78:1657-64] with fluorescence microscopy we are able to observe a phase separated lipid monolayer with varying sub-phase concentrations of nanoparticles. Fluorescently tagged nanoparticles reveal their co-localization at the interface between two monolayer phases. A theoretical model will also be presented to describe this behavior.

1798-Pos Board B708

In Vitro Coralling of a Transmembrane Protein in a Double Cushioned Lipid Bilayer Assembly

Kumud R. Poudel, David J. Keller, James A. Brozik.

In this talk, the characteristic motion associated with a large transmembrane protein (5HT_{3A}*) in a Double Cushion biomimetic assembly, is an assembly that is passivated with a Bovine Serum Albumin layer along with PEG(polyethylene glycol) as the cushion will be presented. These results will be compared to receptors in both planar POPC assemblies and Single cushion assemblies. The main conclusion of this work is that while FRAP recoveries (percent bulk mobility) of these large membrane receptors are quite low in these systems, Single Particle Tracking (SPT) has revealed reasonably large mobile fractions when viewed at much higher spatial resolution and slower time scales. This is especially true for receptors in the 'Double cushion' assemblies. These assemblies display very low FRAP recoveries, but SPT clearly reveal that nearly all receptors are actually quite mobile but are spatially confined to very small corrals. The high mobility of receptors in these coralling domains demonstrates that the strong attractive interactions between the transmembrane protein and solid support have been virtually eliminated and therefore it is reasonable to believe that these receptors will remain active.

1799-Pos Board B709

Divalent Cations Reduce the pH Sensitivity of OmpF Channel Inducing the PKA Shift of Key Acidic Residues

Maria Queralt-Martín, Elena García-Giménez, Salvador Mafé, Antonio Alcaraz.

In contrast to the highly-selective channels of neurophysiology employing mostly the exclusion mechanism, different factors account for the selectivity of large channels. Elucidation of these factors is essential for understanding the permeation mechanisms in ion channels and their regulation in vivo. The interaction between divalent cations and a protein channel, the bacterial porin OmpF, has been investigated paying attention to the channel selectivity and its dependence with the solution pH. Unlike the experiments performed in salts of monovalent cations, the channel is now practically insensitive to the pH, being anion selective all over the pH range considered. Electrostatic calculations based on the available structural data suggest that the binding of divalent cations has two main effects: i) the pKa values of key ionizable groups differ significantly from those of the isolated groups in solution and ii) the cation binding has a decisive impact on the effective electric charge regulating the channel selectivity. A simple molecular model based on statistical thermodynamics provides additional qualitative explanations to the experimental findings that could also be useful for other related systems like synthetic nanopores, ion exchange membranes, and polyelectrolyte multilayers.

1800-Pos Board B710

Structure and Compositional Heterogeneity of Model Skin Lipid Membranes: Site-Directed FRET Approach

Eugene Pashkovski, Nwanyinma Nnodum.

The coexistence of ordered domains found in reconstituted skin lipid membranes using Laurdan GP imaging (I. Plasencia et al., *Biophys. J.* (2007) 93, 3142) suggests that the membranes are laterally heterogeneous on the microscopic length scale. The coexistence of the domains having different water